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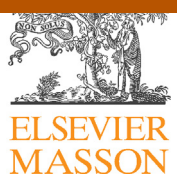

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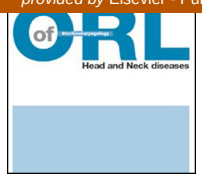

ORIGINAL ARTICLE

BRAF mutation in papillary thyroid carcinoma: Predictive value for long-term prognosis and radioiodine sensitivity

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KEYWORDS
BRAF V600E mutation;
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Summary
Objectives: *BRAF* pV600E mutation is the most common oncogenic event and the most specific mutation for papillary thyroid carcinoma (PTC). Many studies over the last decade have shown a direct relationship between *BRAF* mutation and aggressive tumour characteristics, resulting in poor prognosis. However, several recent studies have suggested that *BRAF* mutation is not associated with poor prognosis of PTC. The present study was designed to evaluate the association between *BRAF* mutation with clinicopathological factors and tumour recurrence.
Material and methods: In this retrospective study, *BRAF* mutation status was examined by direct sequencing on paraffin-embedded tumour specimens from 46 patients undergoing surgery for PTC in our institution from 1985 to 2000. The relationship between *BRAF* mutation and gender, advanced age, extrathyroid extension, multifocal tumour, cervical lymph node metastasis, tumour size and advanced pT stage of PTC and its predictive role for the risk of tumour recurrence were investigated with a median follow-up of 10.1 (±6.5) years.
Results: *BRAF* mutation was detected in 20 of the 46 patients (43.5%) included in the study. No statistically significant correlation was demonstrated between the presence of *BRAF* mutation and the various clinicopathological factors studied. No significant difference in tumour recurrence rate or radioiodine sensitivity was observed between the two subgroups: mutant *BRAF* and wild-type *BRAF*.
Conclusion: Although *BRAF* mutation appears to play a role in local tumour progression, it is not a risk factor for poor prognosis or tumour recurrence in PTC.

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Introduction

Papillary thyroid carcinoma (PTC) is the most common malignant thyroid tumour. The growing incidence of PTC observed over recent decades has been attributed to increasingly reliable and early clinical and ultrasound detection [1].

PTC has an excellent prognosis with 10-year overall survival rates higher than 90% after appropriate treatment [1]. However, 20 to 30% of patients develop local recurrence or distant metastasis and 1% die from their cancer. Identification of this high-risk group at the time of diagnosis would allow more appropriate management and follow-up for these patients.

Among the various oncogenic events identified in PTC, a high prevalence of activating mutation of the gene coding for *BRAF* protein kinase has been observed. This protein is a powerful inducer of the MAP kinase signalling pathway and plays an important role in growth regulation, cell division and proliferation. This mutation is observed in 29 to 83% of PTC. It is specific to PTC and anaplastic carcinomas derived from PTC [2].

The specificity of this mutation and its easy detection make it an attractive genetic marker for the management of PTC. Several studies have demonstrated a significant association between *BRAF* mutation and clinicopathological factors of poor prognosis, although this association remains controversial.

The present study was designed to evaluate the prognostic impact of *BRAF* mutation and its impact on radioiodine resistance in PTC.

Material and methods

Patients

Seventy patients treated for PTC between 1985 and 2000 were retrospectively screened for *BRAF* mutation. Only patients with tumours measuring 10 mm or larger were included. Informative data were available for 46 patients of this population, who were operated in our institution: 45 were treated by one- or two-stage total thyroidectomy and one patient was treated by lobectomy with isthmectomy. This procedure was completed by unilateral or bilateral neck dissection in 17 patients. All patients were treated by adjuvant radioiodine therapy.

Complete remission was defined by the following criteria:

- normal physical examination;
- negative radioiodine whole body scintigraphy after thyroidectomy;
- serum thyroglobulin less than 3 μg/L.

Recurrence was defined by the following criteria:

- elevated serum thyroglobulin;
- suspicious ultrasound image with confirmation of the diagnosis by fine needle aspiration cytology. Thyroglobulin assay in fine needle aspiration cytology rinsing fluid can also be an argument in favour of recurrence;
- positive radioiodine whole body scintigraphy (which may remain negative in the case of radioiodine resistance).

Radioiodine-refractory patients (radioiodine resistance) were defined as:

- patients with no radioiodine uptake or;
- who progressed during the months following radioiodine therapy or;
- who presented persistent disease after a cumulative dose of 600 mCi.

Written consent was obtained from the patents after informing them about use of part of their tumour tissue for research purposes.

Identification of the *BRAF* mutation

Paraffin-embedded tumour blocks from all patients, stored in our institution's pathology laboratory, were reviewed. Five μm sections were stained with haemalun-eosin to localize the most representative tumour zone. Ten μm sections were performed in this zone for tumour genomic DNA extraction. DNA was isolated after protease digestion of the tumour and DNA binding onto a fibreglass filter. Exon 15 was amplified by PCR using a specific primer pair:

Sense primer: 5'-TTCCTTTACTTACTACACCTCAGATATATTTCTT-3'

Antisense primer: 5'-CCAGACAACTGTTCAAACTGATGG-3'

After agar gel migration, the PCR product was purified and submitted to primer extension. This technique is based on the use of fluorescently labelled dideoxynucleotide triphosphate (ddNTP). After PCR and hybridization of the PCR product with an oligonucleotide specific for the 5' flanking sequence of the polymorphism to be detected, a DNA polymerase was used to incorporate the complementary ddNTP of the potentially mutant nucleotide. The fluorescent ddNTP was detected by an ABI 3130xl automatic sequencer (Applied Biosystems). SNaPshot analysis demonstrated mutation of nucleotide 1799, which represents 90% of all known *BRAF* mutations.

Clinical data

To allow comparative statistical analysis, the main clinical data for each patient were collected: age at diagnosis, gender, multifocal tumour, extrathyroid extension, tumour stage (TNM), recurrence.

Statistical analysis

Data are expressed as mean ± standard deviation and percentage. The association between *BRAF* mutation and gender, tumour size, extrathyroid extension, multifocal tumour, presence of cervical lymph node metastasis and tumour stage was evaluated by Fisher's exact test. A Mann-Whitney test was used to compare the presence of *BRAF* mutation and age. A value of $P < 0.05$ was considered to be statistically significant.

Results

Population

Five patients were lost to follow-up and DNA could not be used for *BRAF* mutation screening for two patients.

The mean age of the patients included in the study was 43.2 (± 15.1) years and the mean tumour diameter was 30.6 (± 15.4) mm. Epidemiological and clinicopathological characteristics of the two subgroups are shown in Table 1.

Post-treatment follow-up was comparable in the two subgroups (10 years in the mutant *BRAF* subgroup versus 10.25 years in the wild-type *BRAF* subgroup).

Laboratory study

The T1799A mutation of the *BRAF* gene was demonstrated in 43.5% of the PTC studied. Patients were classified into two subgroups: a mutant *BRAF* subgroup and a wild-type *BRAF* subgroup.

Statistical analysis

The only statistically significant difference between the two subgroups was a greater number of patients with clinical and histological lymph node metastasis in the wild-type *BRAF* subgroup (15%/54%, $p = 0.03$).

Table 2 shows the correlation between *BRAF* mutation and various clinicopathological characteristics of PTC.

No significant correlation was demonstrated between *BRAF* mutation and any of the other clinicopathological criteria studied. At the end of the study, two patients had died from their PTC, one in each of the two subgroups. Survival curves for the two subgroups were identical, but the small number of tumour events did not allow any statistically significant |sions.

Mean follow-up after surgery was 10.1 (± 6.5) years. Eleven patients developed recurrence after a mean postoperative interval of 3.5 (± 3.4) years. Table 3 demonstrates the relationship between recurrence rate and certain prognostic factors.

BRAF mutation was not correlated with an increased risk of tumour recurrence.

Three patients with recurrence harboured a *BRAF* mutation in their primary tumour. Patients with *BRAF* mutation presented more extensive recurrence requiring more aggressive treatment. For 2 out of 3 mutant *BRAF* patients versus 3 out of 8 wild-type *BRAF* patients, the clinical course was marked by multiple local recurrences requiring at least repeat surgery and/or external beam radiotherapy.

One patient of the mutant *BRAF* subgroup (33%) versus one patient of the wild-type *BRAF* subgroup (13%) presented radioiodine resistance at sites of tumour recurrence. The differences between the two subgroups were not significant.

Discussion

Patients of the two subgroups (mutant *BRAF* and wild-type *BRAF*) were comparable and corresponded to the populations reported in the literature concerning differentiated thyroid cancer. Despite the generally favourable prognosis of PTC after initial treatment, 20 to 30% of patients subsequently relapse [3—5]. These patients often require more aggressive complementary management that does not always achieve cure.

Many prognostic factors have been proposed to rapidly identify this high-risk population and consequently limit the morbidity and mortality related to PTC. Advanced age, male gender, large tumour size, extrathyroid extension, multifocal tumour, lymph node and/or distant metastasis have been identified as risk factors for poor prognosis and recurrence [6,7].

A molecular marker such as *BRAF*, that can be easily detected on preoperative fine-needle aspiration cytology, would allow evaluation of the risk of recurrence at the time of diagnosis, and appropriate adjustment of first-line treatment as well as the modalities of post-treatment follow-up.

BRAF mutation is detected in 38 to 83% of PTC with a mean of 49% and plays a central role in initiation of PTC by an early action on carcinogenesis. A similar *BRAF* mutation rate was observed in the present series (44%).

No statistically significant correlation was observed in this study between the presence of a *BRAF* mutation and tumour aggressiveness. Patients with *BRAF* mutation nevertheless appeared to have a slightly higher recurrence rate and recurrences tended to be more aggressive and more extensive in this subgroup. In contrast, lymph node metastases were more frequent among patients of the wild-type *BRAF* subgroup. Radioiodine sensitivity also did not appear to be significantly influenced by *BRAF* mutation, but a tendency towards a certain degree of radioiodine resistance was observed. A limitation of this study was the small sample size, which may have reduced the power of statistical tests.

Some studies [8,9] have shown that *BRAF* mutation is associated with advanced age and extracapsular tumour extension. According to Lupi et al. [10], *BRAF* mutation is correlated with extracapsular tumour extension, lymph node metastases and advanced T stage. In a retrospective series of 219 patients, Xing et al. [11] reported a significant correlation with extracapsular tumour extension, lymph node metastasis, and advanced tumour stage.

The results of this study confirm those of larger published series [12,13]. Ito et al. [13], based on a series of 631 patients with a mean follow-up of 83 months, did not reveal any significant relationship between presence of *BRAF* mutation and age, gender, extrathyroid extension, lymph node and distant metastases, and tumour stage.

The discordant results of these studies have been discussed in several meta-analyses. The review of the literature published by Lee et al. [14], evaluating 12 studies including a total of 1168 patients, reported a significant correlation between *BRAF* mutation and the histological subtype of PTC, extrathyroid extension and advanced tumour stage. In contrast, no significant correlation was observed with age, gender, tumour size and the ethnic group. Kim et al. [15] confirmed the negative impact of *BRAF* mutation on the prognosis of PTC.

These discordant results concerning the prognostic significance of *BRAF* mutation could be due to the inclusion of various histological subtypes of PTC, epidemiological

Table 1 Characteristics of the two subgroups of the study.

	Mutant *BRAF* subgroup	Wild-type *BRAF* subgroup
Total number of patients	20	26
Age at diagnosis (years, mean)	45.8	41.2
Tumour size (mm, mean)	30.7	30.8
Gender (male/female)	2/18	9/17
Multifocal tumour (yes)	3	4
Extrathyroid extension (yes)	7	7
Cervical lymph node metastasis	3	14
Tumour recurrence (yes)	3/11	8/11
Stage (number of patients)		
pT1	6	8
pT2	5	10
pT3	9	5
pT4	0	3

factors, insufficient follow-up, insufficient sample sizes or the use of different methods to detect *BRAF* mutations. These contradictory results could also be explained by the duration of the disease prior to initiation of treatment, which differs for each patient included in the various studies. *BRAF* mutation is frequently detected in papillary microcarcinomas [16—18]. These tumours have an excellent prognosis regardless of *BRAF* status, while a more marked

Table 2 *BRAF* status and clinicopathological characteristics of the 46 patients of the series.

	Total	*BRAF* V600E mutation (+)	*BRAF* V600E mutation (−)	*P* value
Gender				
Male	11	2	9	0.05 (NS)
Female	35	18	17	
Age at diagnosis (years), mean	43.2 (± 15.1)	45.8	41.2	0.18 (NS)
Age < 45 years	20	10	10	0.31 (NS)
Age < 45 years	26	10	16	
Tumour size (mm)				
< 2 cm	11	5	6	0.57 (NS)
≥ 2 cm	35	15	20	
Extrathyroid extension				
Yes	14	7	7	0.39 (NS)
No	32	13	19	
Multifocal nature				
Yes	7	3	4	0.65 (NS)
No	39	17	22	
Cervical lymph node metastasis				
Yes	17	3	14	0.03 (IC)
No	29			
Stage				
pT1	14	6	8	0.17 (NS)
pT2	15	5	10	
pT3	14	9	5	
pT4	3	0	3	

NS: not significant; IC: inverse correlation.

Table 3 Association between clinicopathological factors and recurrence rate.

	Sample size	Recurrence rate (%)	*P* value
Age at diagnosis			
< 45 years	26	30.8	0.18 (NS)
≥ 45 years	20	15	
Gender			
Male	11	27.3	0.53 (NS)
Female	35	22.9	
Extrathyroid extension			
Yes	14	35.7	0.19 (NS)
No	32	18.8	
Multifocal nature			
Yes	7	28.6	0.54 (NS)
No	39	23.1	
pT stage			
T1	14	14.3	0.03
T2	15	26.7	
T3	14	14.3	
T4	3	0	
BRAF status			
Wild-type	26	72.7	0.19 (NS)
Mutant	20	27.3	

NS: not significant.

difference in prognosis is observed between more advanced PTC according to the presence or absence of *BRAF* mutation. This heterogeneity can affect the probability of demonstrating correlations between *BRAF* mutation and prognosis of PTC.

BRAF mutation occurs early in the course of PTC carcinogenesis, but does not appear to be sufficient to confer a more aggressive phenotype. *BRAF* mutation could be considered to predispose tumour cells to the development of other oncogenic events, leading to a certain degree of loss of differentiation and a poorer prognosis.

BRAF and tumour recurrence

The individual risk of tumour recurrence is evaluated by taking into account the patient's age at the time of diagnosis, the histological subtype of PTC, the extent of tumour invasion and the extent of surgical resection. The disadvantage of this approach is that it cannot be applied preoperatively. Very few studies have analysed the correlation between *BRAF* mutation and the outcome of patients with PTC with long-term postoperative follow-up.

In this series, with a median follow-up of more than 10 years:

- the presence of *BRAF* mutation in the primary tumour was not associated with an increased risk of recurrence. However, the survival curves of the two subgroups could not be compared due to the small sample size of this study;
- patients with *BRAF* mutation presented more extensive recurrence, requiring more aggressive treatments comprising at least repeat surgery and/or external beam radiotherapy compared to the wild-type *BRAF* subgroup, but the difference was not statistically significant;
- the estimated recurrence rate was 24% with no significant difference between the two subgroups;
- 98% of patients underwent total thyroidectomy, all received at least one dose of radioiodine and neck dissection was performed during the initial surgical management in 37% of cases. These results could be explained by more extensive initial surgical resection and adjuvant radioiodine therapy that limit local recurrence.

Elisei et al. [19] compared the survival curves of patients expressing wild-type *BRAF* and those with *BRAF* mutation with a follow-up of 15 years. Tumour recurrence and cancer-specific mortality rates were higher for *BRAF* mutation patients.

The results of the present study are similar to those reported by Cañadas Garre et al. [20], who concluded on the absence of any significant correlation between *BRAF* mutation and tumour recurrence, progressive disease and the use of aggressive adjuvant therapy.

The quality of the initial resection and the use of adjuvant radioiodine therapy could compensate for the negative effect of *BRAF* mutation on the course of PTC [6,21].

In this series, the lymph node metastasis rate was higher among patients in the wild-type *BRAF* subgroup. This statistically significant difference is in contradiction with the results of the meta-analysis by Lee [14], in which the lymph node metastasis rate was non-significantly higher in the mutant *BRAF* subgroup. This difference could be due to the small sample size of our study, as well as ethnic difference,

as one half of the patients included in Lee's meta-analysis were of Asian origin.

BRAF and radioiodine resistance

An association between *BRAF* mutation and the expression of certain genes involved in radioiodine metabolism has been proposed as a possible explanation for treatment failures and recurrence of PTC. *BRAF* mutation appears to be associated with radioiodine resistance, responsible for a higher recurrence rate. Abnormalities of tumour iodine metabolism appear to be related to decreased expression of the sodium-iodine symporter (NIS) and thyroperoxidase. Some teams [22—24] have shown that *BRAF* mutation alters the transcription of the gene encoding NIS and decreases its membrane expression, while other teams [25] failed to demonstrate any link between *BRAF* mutation and NIS expression, suggesting that decreased NIS expression is not the only mechanism responsible for radioiodine resistance.

In this study, no significant difference in radioiodine sensitivity was observed between the mutant *BRAF* and wild-type *BRAF* subgroups, probably because of the small sample size.

Conclusion

This study analysed the correlations between *BRAF* mutation and clinicopathological characteristics as well as the long-term prognosis of PTC. No significant correlation was observed between *BRAF* mutation and the various clinicopathological factors known to be associated with poor prognosis of PTC. *BRAF* mutation was also not correlated with an increased tumour recurrence rate after first-line treatment or with radioiodine sensitivity. A multicentre study based on a larger sample size with long-term follow-up should be conducted to clarify the role of *BRAF* mutation in PTC tumour aggressiveness.

Disclosure of interest

The authors have not supplied their declaration of conflict of interest.

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